AD-A135 268 A BIODEGRADABLE AND PROTEOLIPID BONE REPAIR COMPOSITE (U) ARMY INST OF DENTAL RESEARCH WASHINGTON DC J O HOLLINGER 10 NOV 83 1/1

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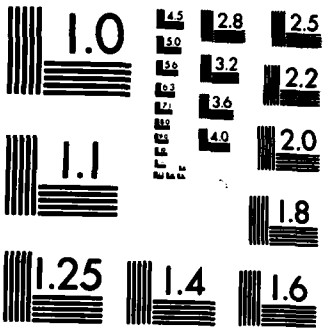

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Polylactide, Ploy glycolide, Acidic Phospholipid, Biodegradable, Biocompatible

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A composite bone repair agent consisting of the copolymer polylactide and polyglycolide was completed with an acidic phospholipid. The resulting material was biodegradable and biocompatible. This material was pieced into endochondral defects in rats. Histochemical and histomorphometric data which indicated that the defects treated with the bone repair composite healed more rapidly than nontreated control wounds.

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J. Holliger

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A Biodegradable And Proteolipid Bone Repair Composite

INTRODUCTION:

Many different types of materials have been used for the purpose of repairing, replacing, or augmenting bone. Autografts are the favored modality of treatment used by orthopedic and maxillofacial surgeons.[1] Unfortunately, there are numerous disadvantages associated with autogenous grafting, such as unpredictable results, failure rates ranging from 13% to 30%, inability to recover sufficient autogenous bone, technical inconvenience, and trauma to the patient as a consequence of a second surgical site.[2,3] Allografts and alloimplants also are plagued with many problems.[4,5] Derivatives of bone, such as demineralized bone and collagen gels have been employed with mixed results. The use of ceramics for bone repair or augmentation has been described extensively.[6,7] This class of agents has a limited orthopedic utility. Ceramics may be useful for the treatment of some types of alveolar bone loss in periodontal disease. Biopolymers known as poly-α-hydroxy acids (a class of polyesters) have garnered considerable attention in the medical and dental fields in the past ten to fifteen years. The poly-α-hydroxy acids known as polygloclic acid (PGA) and polylactic acid (PLA) were initially formulated and described as biodegradable suture materials.[8,9,10] They are commercially available as Dexon® and Vicryl®. Different formulations of the PLA and PGA also have been used experimentally for osseous repair and reconstructive procedures.[11,12,13,14,15,16]

Considerable attention has been focused on the type II matrix vesicle for initiating calcification.[17,18,19] Intensive investigation of the structure of the matrix vesicle has revealed that its trilaminar membrane possesses a high content of acidic phospholipid.[20,21] It has been shown that a protein-acidic

phospholipid complex similar to that of the matrix vesicle can induce hydroxy apatite formation both *in vitro* and *in vivo*.[22,23,24] The formulation of the protein-acidic phospholipid complex that was used by Hollinger[24] has a paste-like consistency that is unsatisfactory for most types of bony wound applications, such as when rigid fixation is required or when expansive discontinuity defects must be repaired. It was the purpose of this study, therefore, to develop a composite bone repair material that would have utilitarian application. The combination of the biodegradable polyesters of PLA and PGA with a protein-acidic phospholipid component were conceived for such a purpose.

METHODS and MATERIALS:

The experimental bone repair materials were prepared according to the flow diagrams (1, 2, 3, and 4). Bone repair agents were evaluated according to Tables I and II. All tissues were prepared for histomorphometric assessment described by Hollinger[14] using an Image Analysis System (IAS) (diagram 5). The possible number of bony wound healing measurements that could be generated for this study are presented in Table III.

DATA ANALYSIS:

Data from the analyses of the same variable (i.e., bony trabecula) from the same treatment and temporal groups were combined, yielding a pooled mean value based upon 2,000 measurements. A standard deviation and a standard error of the mean were computed for each set of the 2,000 measurements for each value according to the formulae[25,26] The pooled mean for each variable (based upon 2,000 fields) plus and minus its standard error of the mean was then used to construct a histogram. A trio of sets (i.e., A_3, B_3, C_3) representing treatment and temporal groups were arranged along the abscissa and the corresponding units or percentage appeared along the ordinate.

A two-way analysis of variance was used to examine and to test the effects of (1) the treatment groups (differences between the three treatment groups analyzed over the six time periods) and (2) time periods (differences between the six time periods averaged over the three treatment periods). Effects having an observed significance level (p-value) equal to or less than 5% (based on the appropriate F-test value which was derived by performing the two-way analysis of variance) were considered to be statistically significant. Differences between the pooled means of any two treatment groups were tested by partitioning the overall difference between treatment group variability, with variability being equivalent to the sum of squares. Specific comparisons were then made between treatments.

RESULTS:

Statistical and Histomorphometric:

The statistical evaluation based upon the analyses of 180,000 possible histomorphometric measurements is summarized in Table IV. Histograms I-IV represent a sample of the histograms derived from the data for each of the variables that were analyzed with the IAS.

Histological:

The overall trends in healing frequently displayed only subtle visual differences when viewed histologically. Photomicrographs of selected stages of repair can be observed in Figures 1-6.

All treatment groups displayed the typical patterns of osseous wound healing. There was often equivocal evidence that histologically evaluated wound healing might have been superior for one type of treatment class than in another; however, when histomorphometric assessments and statistical analyses were preformed, contrasts were extracted that frequently proved to be

significantly different.

Histological evaluation seemed to indicate that the patterns of healing consisted of more reparative elements in the copolymer-proteolipid (group A) treated sites at an earlier period in time than either the plain copolymer (group B) or control (group C) sites. Further, group B treated wounds appeared to possess more of the elements of osseous repair at the same time periods than group C treated wounds. It did not appear that the presence of the composite implant material deterred bony healing, which centripetally manifested. There was no callus formation and no evidence of an adverse inflammatory response engendered from the implants from either groups A or B.

DISCUSSION:

Materials such as bone grafts and implants, collagen gels, ceramics, bone derivatives, and biopolymers are some of the many agents which have been employed by orthopedic and maxillofacial surgeons for initiating osseous repair or for replacing bone. Failure to achieve beneficial results with these materials has not been necessarily a consequence of imprudence; but rather, due to deficiencies inherent to the repair and replacement agents. A combination of the biopolymers PLA and PGA in conjunction with the particular proteolipid described, appears to offer considerable promise as an alternative to the more common, conventional materials.

Hollinger[24] prepared a specific proteolipid for implantation into endochondral bone defects in rats. The healing response elicited by the proteolipid exceeded that of the control sites. Hollinger[24] suggested that the material estabished a unique chemical environment conducive to calcium and phosphate precipitation, nucleation, and subsequent crystal growth. Moreover, the implication was that the locally introduced proteolipid was tantamount to surrogate

extracellular matrix vesicles, the structure whose limiting membrane is heavily endowed with an acidic phospholipid component. Their extremely critical function in the calcification scheme has been described at length.[27,19,28]

The breakdown of the biopolymers of PLA and PGA occurs by nonspecific hydrolytic scission that results in the generation of lactic acid and glycolic acid residues.[29,30,31] The lactic acid becomes incorporated in the TCA cycle and is excreted by the lungs as CO_2. Glycolic acid dimers, trimers, etc., are enzymatically degraded by esterases and carboxy peptidases[32] and are converted to monomers of glycolic acid which either can be excreted in the urine or enzymatically converted by glycolate oxidase to glyoxylate.[29] This moiety reacts with glycine transaminase and the glycine that is produced can be used for synthesis of serine, which can be employed in the TCA cycle after transformation into pyruvate.[33,34]

Hollinger[35] has speculated that the positive bone healing response engendered in experimental animals from the copolymer of PLA and PGA may be a consequence of several factors. The linear polyester macromolecular structure could act as a matrix, trellis, or foundation upon which bony reparative elements may be consolidated. Further, a possible consequence of the degradation of the copolymer could be that the pH of the local environment was altered and the potential inhibitors to calcification (proteoglycans, glycosaminoglycans) were debilitated and rendered ineffectual. The pH changes and the organic monomeric acid residues interaction with host organic matrix could function as a mechanism engendering release from the matrix of certain polypeptides, such as bone morphogenetic protein and human skeletal growth factor.[36,37] These factors have been speculated as being agents capable of increasing both osteoblast progenitor cell proliferation and subsequent bone formation rate.

REFERENCES

1. Tuli SM, Singh AD, "The Osteoinductive Property of Decalcified Bone Matrix," J Bone Joint Surg, 60B:116-123, 1978.

2. Tuli SM, Gupta KB, "Bridging of Large Chronic Osteoperiosteal Gaps by Allogeneic Decalcified Bone Matrix Implants in Rabbits," J Trauma, 21(10), pp. 894-898, 1981.

3. Urist MR, "Practical Applications of Basic Research on Bone Graft Physiology," Am Acad Orthop Surgs, 25:1-26, 1976.

4. Burchardt H, Enneking WF, "Transplantation of Bone," Surg Clin North Am, 58:403-422, 1978.

5. Friedlander GE, Sell KW, Strong DM, "Bone Allograft Antigenicity in an Experimental Model and in Man," Acta Med Pol, 19:197-205, 1978.

6. de Groot K, Bioceramics of Calcium Phosphate, CRC Press, 1983.

7. Jarcho M, "Calcium Phosphate Ceramics as Hard Tissue Prosthetics," Clin Orthop, 94:218-305, 1981.

8. Cutright DE, Hunsuck EE, "Tissue Reaction to the Biodegradable Polylactic Acid Suture," Oral Surg, 31:134-139, 1971.

9. Frazza E, Schmitt EE, "The Value of Absorbable Suture," J Biomed Mater Res, 1:43-58, 1971.

10. Kulkarni RK, Pani KC, Neuman C, and Leonard F, "Polylactic Acid for Surgical Implants," Arch Surg, 93:839-843, 1966.

11. Cutright CC, Hunsuck EE, and Beasley JD, "Fracture Reduction Using a Biodegradable Material, Polylactic Acid," J Oral Surg, 29:393-397, 1971.

12. Cutright CC, Hunsuck EE, "The Repair of Fractures of the Orbital Floor Using a Biodegradable Material, PLA." J Oral Surg, 33:28-34, 1972.

13. Getter L, Cutright DE, Bhaskar SN, Augsburgh JK, "A Biodegradable Intra-

osseous Appliance in the Treatment of Mandibular Fractures," J Oral Surg, 30:344-348, 1972.

14. Hollinger JO, "Facilitation of Osseous Healing by a Proteolipid Copolymer Material," Ph.D. dissertation, 1983.

15. Nelson JF, Stanford HG, Cutright DE, "Evaluation of a Comparison of Biodegradable Substances as Osteogenic Agents," Oral Surg, 43:836-843, 1977.

16. Olson RJ, Roberts DL, and Osborn DB, "A Comparative Study of Polylactic Acid, Gelfoam, and Surgicel in Healing Extraction Sites," Oral Surg, 53:276-284, 1982.

17. Anderson HC, "Introduction to the Second Conference on Matrix Vesicle Calcification." Metab Bone Dis Rel Res, 1:83-87, 1978.

18. Muhlrad A, Bab A, Deutsch D, Sela J, "Occurrence of Actin-like Protein in Extracellular Matrix Vesicles," Calcif Tissue Int, 34:376-381, 1982.

19. Wuthier RE, "A Review of the Primary Mechanism of Enchondral Calcification with Special Emphasis on the Role of Cells, Mitochondria and Matrix Vesicles," (Sec. III, Basic Science and Pathology), Clin Orthop 169, 219-242, 1982.

20. Vogel JJ and Boyan-Saylers BD, "Acidic Lipids Associated with the Local Mechanism of Calcification, A Review," Clin Orthop, 118:230--241, 1976.

21. Wuthier RE, "Lipid Composition of Isolated Epiphyseal Cartilage Cells, Membranes, and Matrix Vesicles," Biochim Biophys Acta, 409:128-139, 1975.

22. Boyan-Saylers BD, Boskey AL, "Relationship Between Proteolipids and Calcium-Phospholipid-Phosphate Complexes in Bacterionema matruchotii," Calcif Tissue Int, 30:167-174, 1980.

23. Ennever J, Boyan-Saylers B, Riggan JL, "Proteolipid and Bone Matrix Calcification in vitro," J Dent Res, 56:967-970, 1977.

24. Hollinger JO, "In vivo Calcification Induced by a Proteolipid Complex (lysozyme-acidic phospholipid)," Biomat Med Dev Art Org, 10(2): 71-83, 1982.

25. Dunn OJ, Basic Statistics: A Primer for the Biomedical Sciences, John Wiley & Sons, Inc., New York, pp. 38-44, 1967.

26. Sokal RR, Rohlf RJ, Biometry, WH Freeman and Co, San Francisco, pp. 57-62, 1969.

27. Vogel JJ, Boyan-Saylers BD, and Campbell MM, "Protein-Phospholipid Interactions in Biologic Calcification," Metab Bone Dis and Rel Res, 1:149-153, 1978.

28. Yaari AM, Shapiro DM, "Effect of Phosphate on Phosphatidyl Serine-Mediated Calcium Transport," Calcif Tissue Int, 34:43-48, 1982.32.

29. Bovey FA, Winslow FR, Macromolecules: An Introduction to Polymer Science, Academic Press, New York, 1979.

30. Chu CC, "The in vitro Degradation of Poly (glycolic acid) Sutures - Effect of pH," J Biomed Mater Res, 15:895-804, 1981.

31. Kronenthal RL, "Biodegradable Polymers in Medicine and Surgery," in Polymers in Medicine and Surgery, Kronenthal RL, Oser Z, and Martin E, (ed), Plenum Publishing Corp, New York, pp. 119-137, 1975.

32. Williams DF, Mort E, "Enzyme Accelerated Hydrolysis of Polyglycolic Acid," J Bioengin, 1:231-238, 1977.

33. Dunne R, Personal Communication, 1983.

34. White A, Handler P, Smith EL, Hill RL, Lehman IR, "Collagen," in Principles of Biochemistry, 5th edition, McGraw-Hill Book Co, New York, pp. 1135-1143, 1979.

35. Hollinger JO, "Preliminary Report on the Osteogenic Potential of a Biodegradable Copolymer of Polylactide (PLA) and Polyglycolide (PGA)," J Biomed Mater Res, 17: 71-82, 1983.

36. Farley JR, Baylink DS, "Purification of a Skeletal Growth Factor From Human Bone," Biochemistry, 21:3508-3513, 1982.

37. Urist MR, "Bone Transplants and Implants," in Fundamental and Clinical Bone Physiology, MR Urist, (ed), JB Lippincott Company, Philadelphia, pp. 331-368, 1981.

TABLE I

Organization of Treatment and Temporal Groups

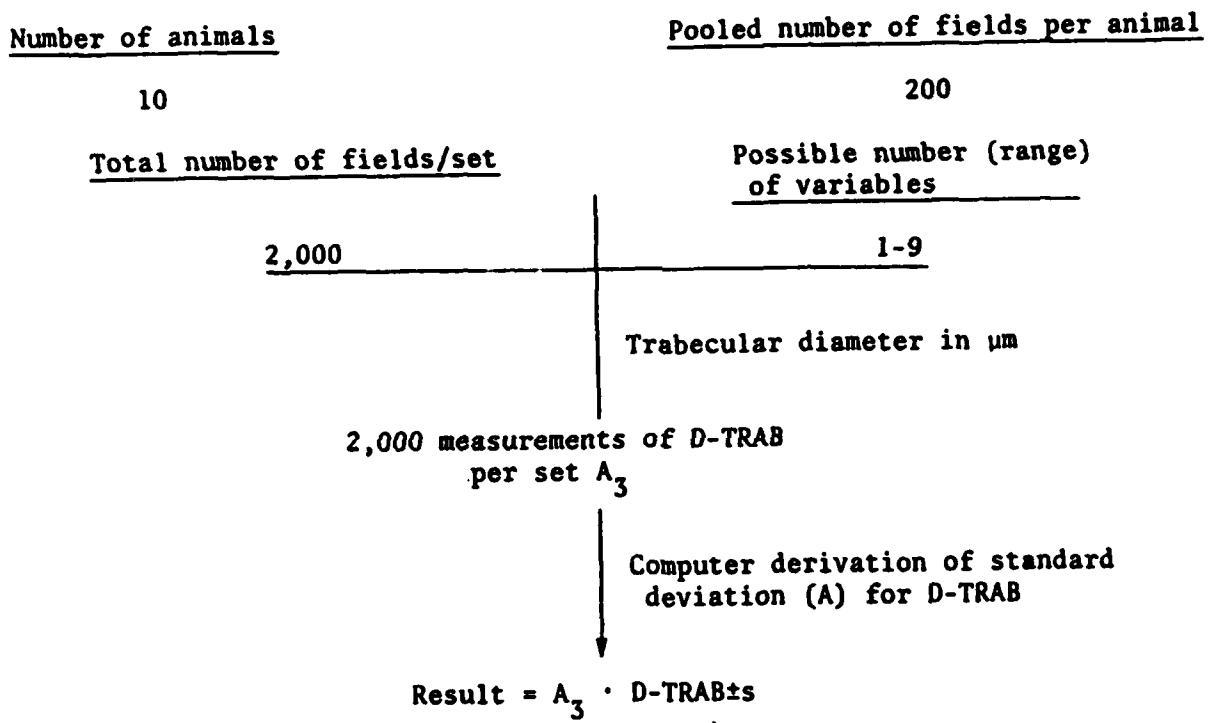
Treatment group (Each treatment group had 10 animals per temporal group)	A (50:50 PLA: PGA-DPI-L)	B 50:50 PLA: PGA –	C Control
Temporal group (Number of days post-treatment that animals were sacrificed)			
3	10	10	10
7	10	10	10
14	10	10	10
21	10	10	10
28	10	10	10
42	10	10	10
Total number of animals per treatment group	60	60	60

PLA:PGA = Polylactic acid: Polyglycolic acid

DPI-L = Diphosphoinositide - Lysozyme

TABLE II

Organization of Data for the Variable Trabecular Diameter (D-TRAB) from Treatment Group A, Temporal Group 3 Days



Key:

T = Temporal group (3, 7, 14, etc.) in days

Tx = Treatment group (A, B, C)

Set = (Tx) (T) = A_3, B_3, C_3, A_7, etc.

s = Standard deviation

TABLE III

Example of Possible Number of Variables
from Treatment Groups A, B, and C

Temporal groups (T) in days	3	7	14	21	28	42	
Pooled number of fields	200	200	200	200	200	200	
Number of animals per T	10	10	10	10	10	10	
Total number of fields	2,000	2,000	2,000	2,000	2,000	2,000	
						Grand total	12,000

Explanation:

1. There were ten animals per temporal group (T).

2. There were 200 fields measured per animal; therefore, 2,000 fields were measured per T (200 X 10 = 2,000).

3. The possible range of derived variables was from one to nine with a mean of five; therefore, 10,000 pieces of information could be computed per T (2,000 X 5. = 10,000).

4. The term set can be defined as a treatment group (Tx) at a particular time (that is, a T of 3, 7, 14, 21, 28, or 42).

5. A grand total (average) of 180,000 measured values could, therefore, be derived (10,000 X 18 = 180,000).

TABLE IV

Results of the Two-Way Analysis of Variance of Sum of Squares

	Time derived values		Sum of squares		Treatment derived values	
	F-test	p	Treatment	Time	F-test	p
Variable▲						
VV	65.08●●		24,782	192,311	21.32●	
Treatment contrasts▲▲						
A vs. B		**				**
A vs. C		**				**
B vs. C		**				**
D-TRAB	179.93●●		2,322	38,603	27.02●	
A vs. B		**				**
A vs. C		**				**
B vs. C		**				**
TH-OS	127.79●●		205	1,958	33.34●	
A vs. B		**				*
A vs. C		**				**
B vs. C		**				**
VV-OS	85.03●●		10.2	205.7	10.21●	
A vs. B		**				**
A vs. C		**				*
A vs. C		**				

TABLE IV (Con't.)

Results of the Two-Way Analysis of Variance of Sum of Squares

	Time derived values		Sum of squares		Treatment derived values	
	F-test	p	Treatment	Time	F-test	p
Variable ▲						
OBI	53.99●●		5,706	123,364	6.85●	
Treatment contrasts ▲ ▲						
A vs. B		**				
A vs. C		**				**
B vs. C		**				*
OB%	53.99●●		157	4,480	6.85●	
A vs. B		**				
A vs. C		**				**
B vs. C		**				*
OCI	7.55●●		6.8	1,857.3	2.43●	
A vs. B		**				
A vs. C		**				
B vs. C		**				
L-TOT%	247.51●●		0.51	280.22	1.02●	
A vs. B		**				
A vs. C		**				
B vs. C		**				

TABLE IV (Con't.)

Results of the Two-Way Analysis of Variance of Sum of Squares

Variable▲	Time derived values F-test	p	Sum of Squares Treatment	Time	Treatment derived values F-test	p
VV-MAR	495.59●●		9,815	512,756	23.73●	
Treatment contrasts▲▲						
A vs. B		**				**
A vs. C		**				**
B vs. C		*				**

Key: ▲ = Variables described in section III. J.
** = p <0.01
* = p <0.05
●● = F confidence level 99%
● = F confidence level 95%
▲▲ A = Copolymer plus proteolipid
B = Copolymer
C = Control

(VV) = Volumetric density of bone

(D-TRAB) = Mean trabecular diameter

(TH-OS) = Mean width of osteoid

(VV-OS) = Volumetric density of osteoid

(OBI) = Osteoblast index

(OB%) = Fraction of total trabecular surface covered by osteoid

(OCI) = Osteoclast index

(L-TOT%) = Fraction of trabecular surface exhibiting resorptive lacunae

(VV-MAR) = Volumetric density of marrow

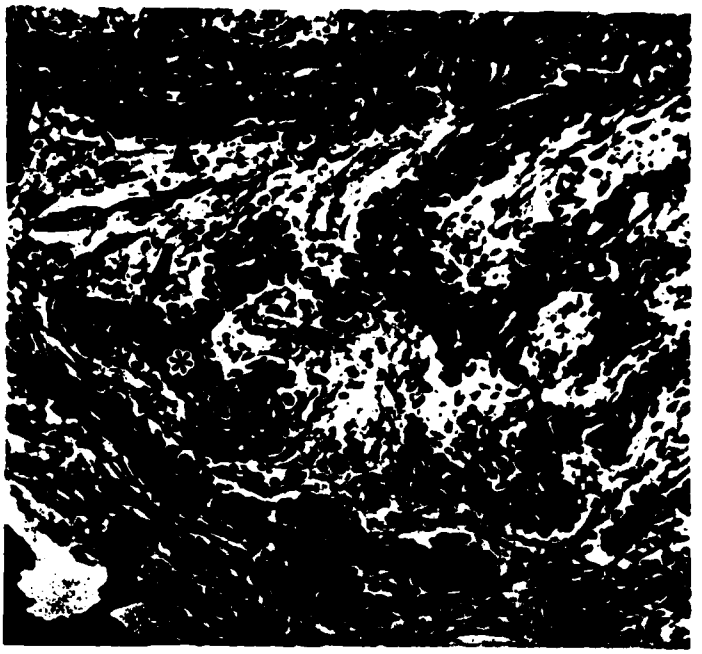


Fig. 1. Copolymer-proteolipid (Group A) treated wound site at three days. Bone trabeculae (*) hapazardly oriented and rimmed with osteoid and osteoblasts.

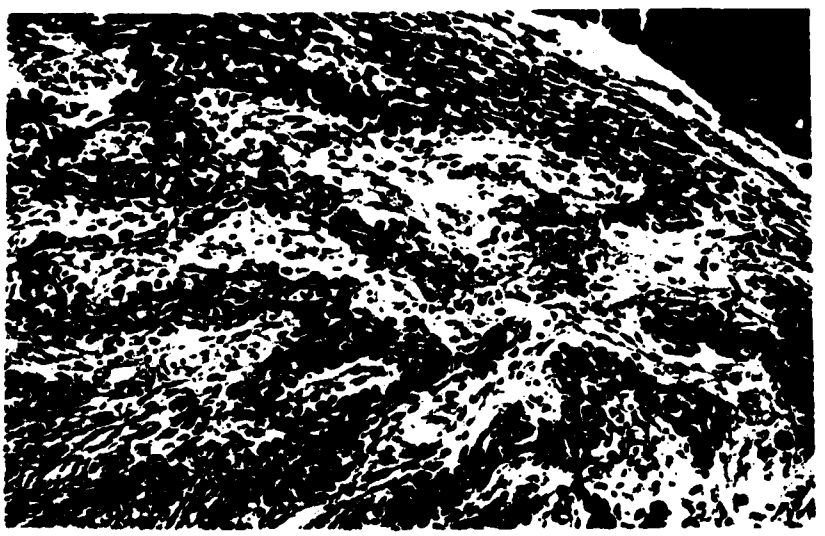


Fig. 2. Copolymer (Group B) treated wound site at three days. Area where osteoid being deposited and less calcified materials present than for Group A.

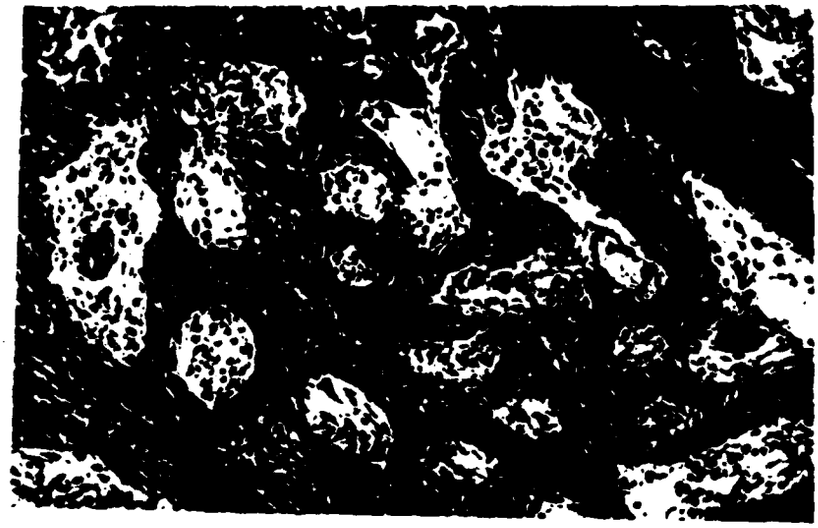


Fig. 3. Control (Group C) wound site at three days. Swirling pattern of fibrous connective tissue with numerous fibro blasts and occasional osteoid and bony trabeculae formation.

Fig. 4. Group A twenty-eight days with numerous trabeculae that are coalescing.

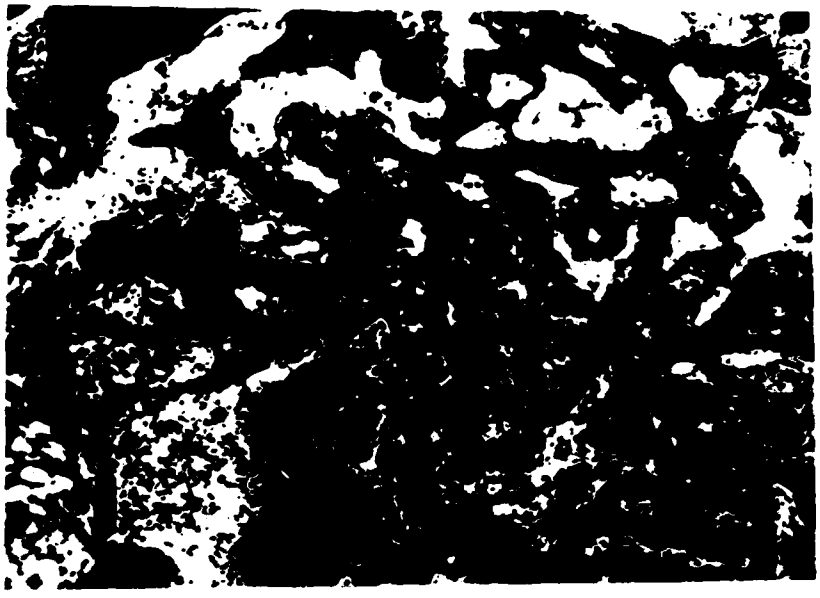


Fig. 5. Group B twenty-eight days demonstrating an area of active repair adjacent to the intact cortex.

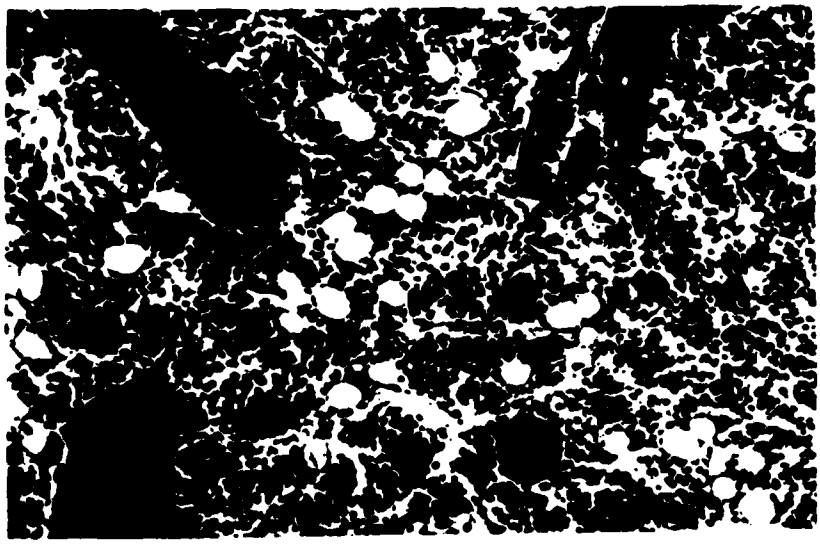
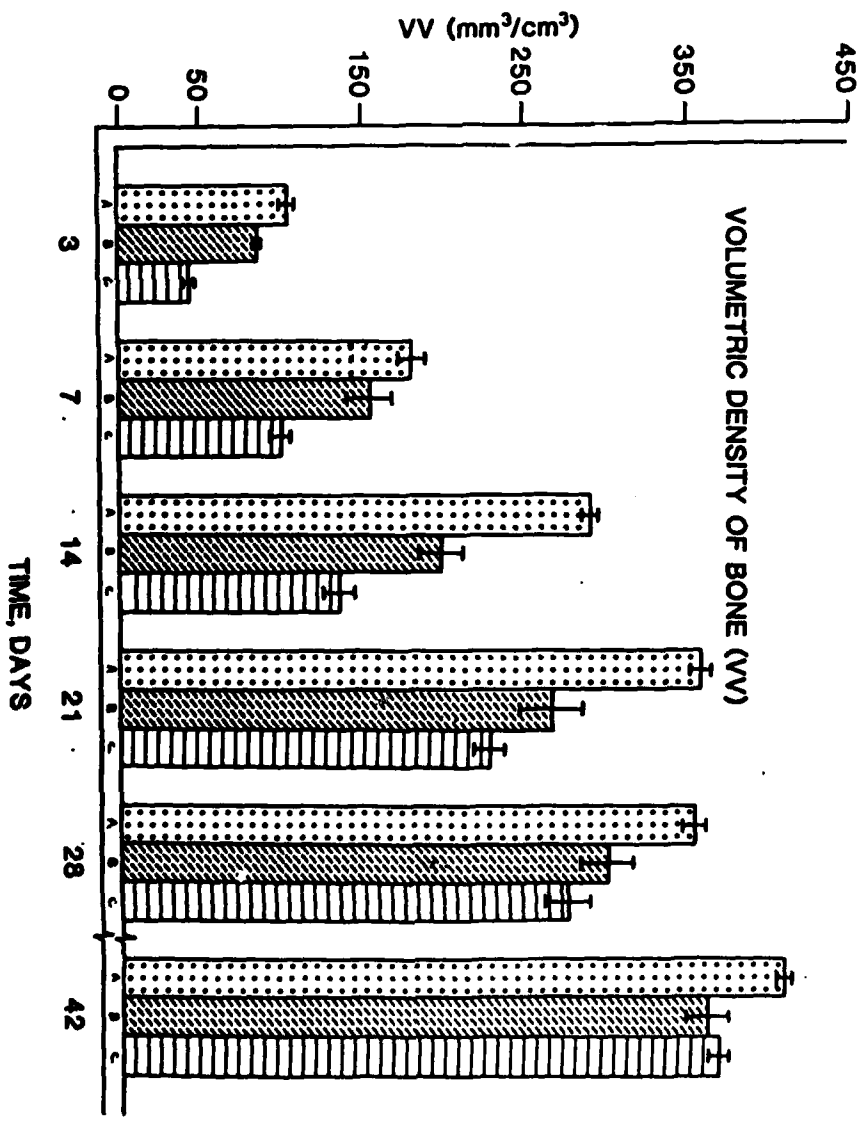


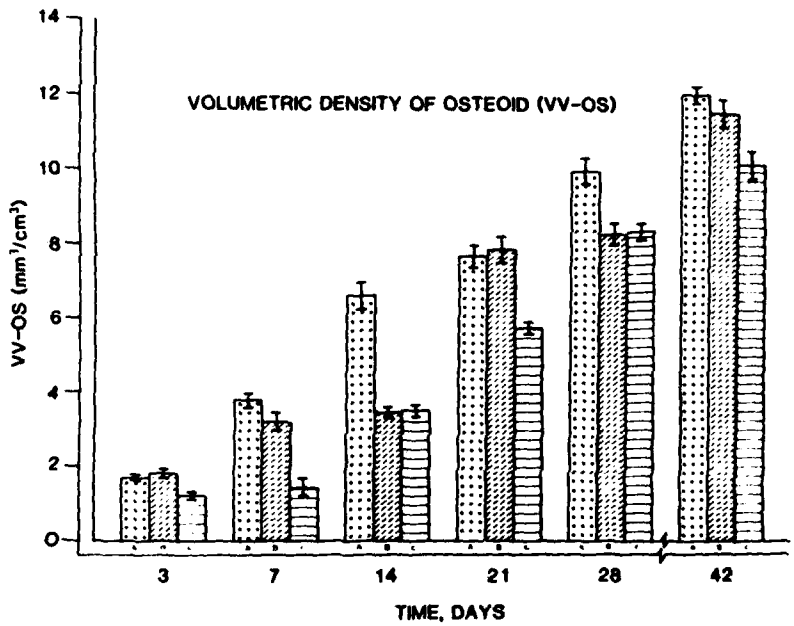
Fig. 6. Group C at twenty-eight days displaying normal elements of wound repair such as trabeculae osteoid, and osteoblasts.

Histogram I

Vomumetric Density of Bone



HISTOGRAM II



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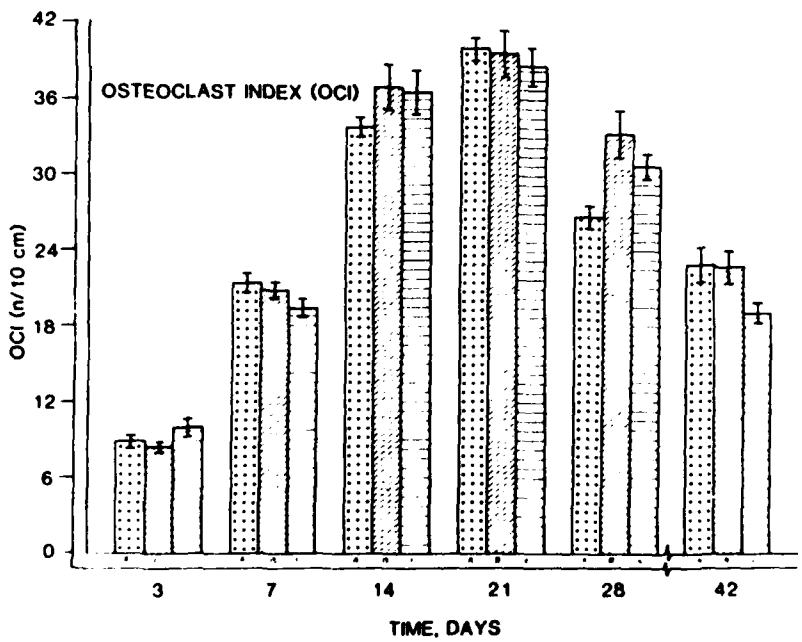


Histogram IV

Volumetric Density of Marrow

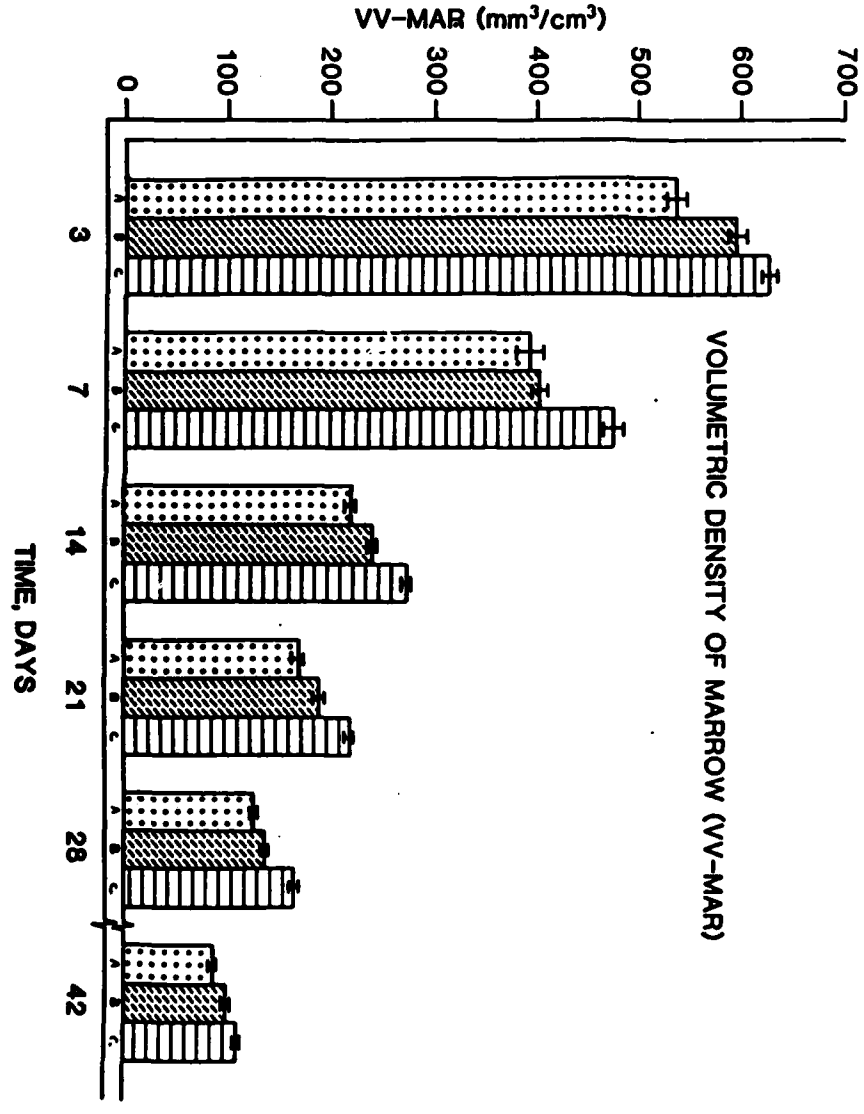
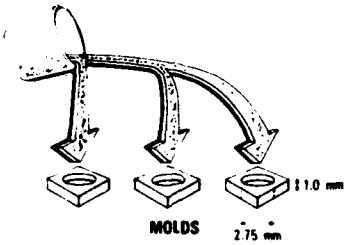


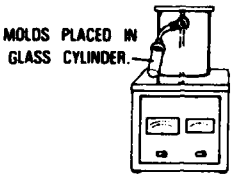
DIAGRAM I

(FABRICATION – CONTINUED)

SOUP POURED INTO TEFLON® MOLD.



MOLDS PLACED INTO LYPHOLYZER
24 HOURS X AMBIENT TEMPERATURE.

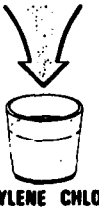


COPOLYMER IMPLANTS RETRIEVED
AND STORED IN DESICCATOR.

DIAGRAM II

FABRICATION OF COPOLYMER IMPLANT

50 : 50 POLYLACTIDE : POLYGLYCOLIDE COPOLYMER



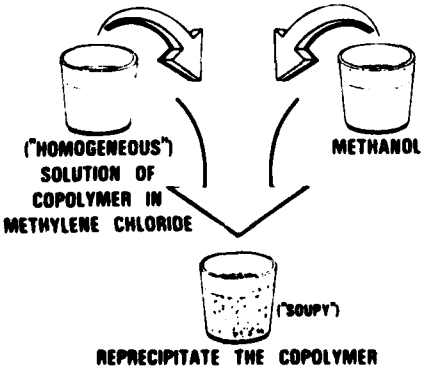


DIAGRAM III

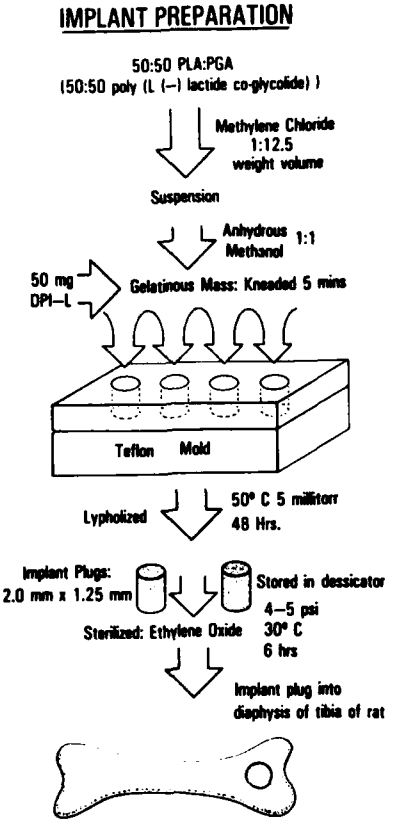


DIAGRAM IV

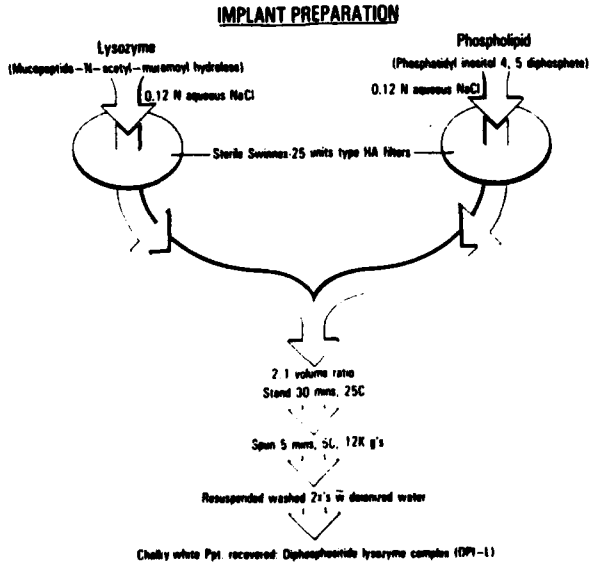
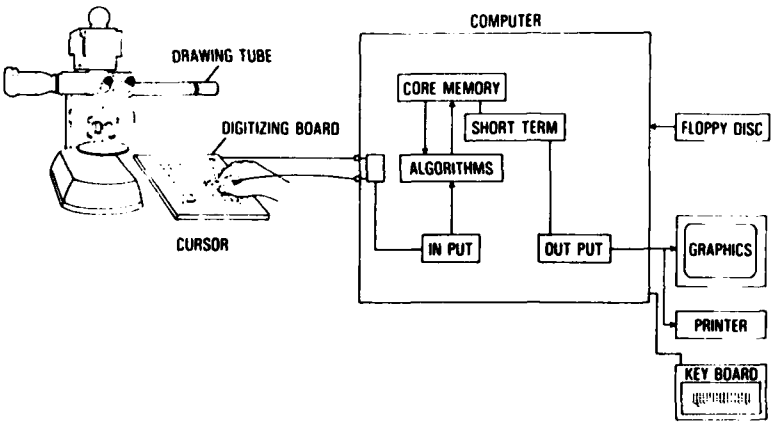
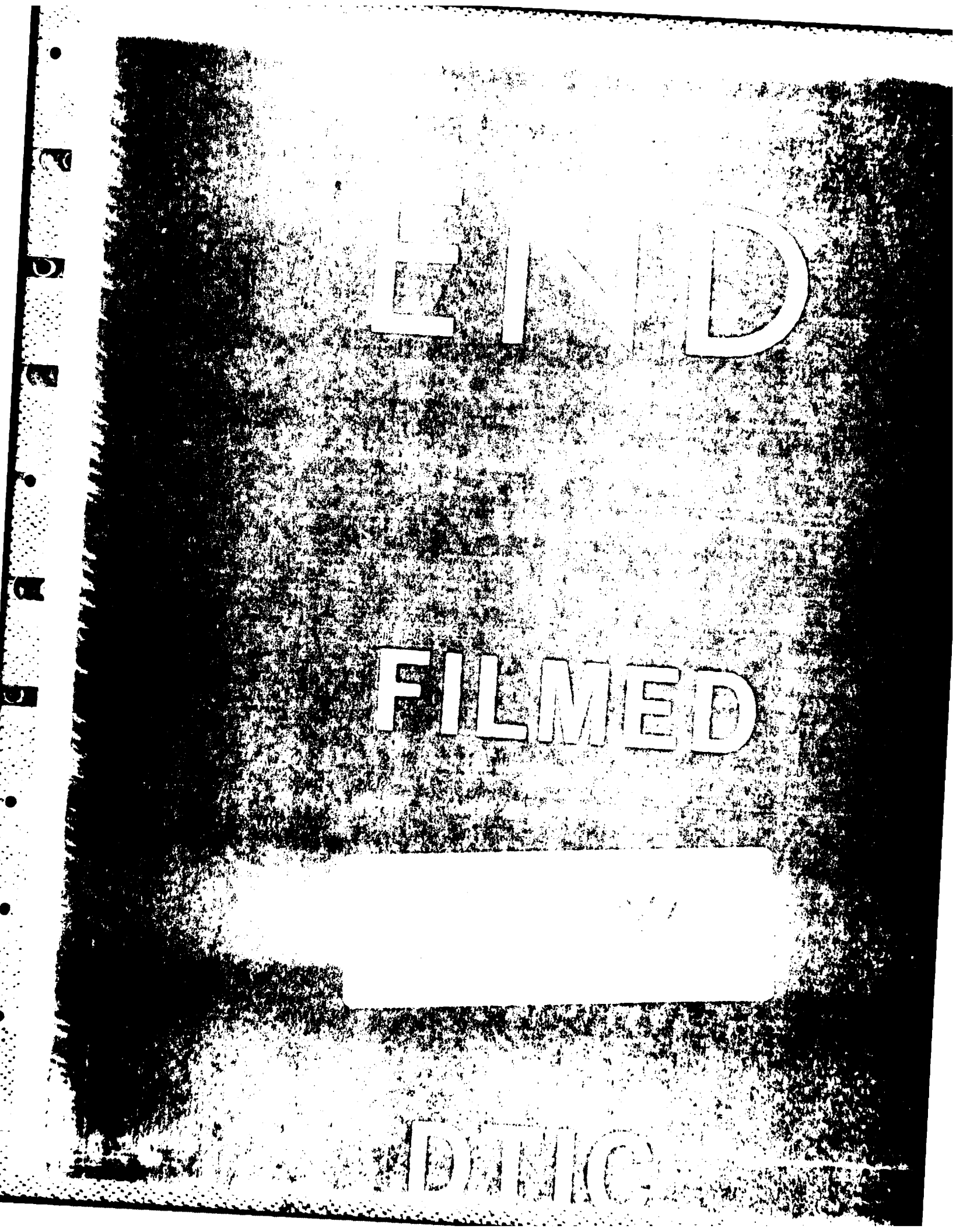


DIAGRAM V



CELLULAR STRUCTURE MAY BE TRACED ONTO A DIGITIZING TABLET WITH A LIGHT CURSOR WHILE SIMULTANEOUSLY OBSERVING THE TISSUE SPECIMEN THROUGH THE MICROSCOPE.


END
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